Human SARS-CoV-2 Spike (trimer) Ig Total ELISA Kit

Catalog Number BMS2323 (96 tests), BMS2323TEN (10 × 96 tests)

Pub. No. MAN0019615 Rev. C.0 (32)

Product description

The Human SARS-CoV-2 Spike (trimer) Ig Total ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of Human SARS-CoV-2 Ig total in serum and plasma.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No.	
	BMS2323 (96 tests)	BMS2323TEN (10 × 96 tests)
Human SARS-CoV-2 Ig total High Control, lyophilized	1 vial	10 vials
Human SARS-CoV-2 Ig total Calibrator, lyophilized	1 vial	10 vials
Human SARS-CoV-2 Ig total Low Control, lyophilized	1 vial	10 vials
Human SARS-CoV-2 Spike (trimer) Coated Plate	1 plate	10 plates
Human SARS-CoV-2 Ig total HRP Conjugate (100X)	0.120 mL	10 × 0.120 mL
Assay Buffer (20X)	50 mL	5 × 50 mL
Wash Buffer Concentrate (20X)	50 mL	6 × 50 mL
Stabilized Chromogen (Tetramethylbenzidine)	15 mL	10 × 15 mL
Stop Solution	15 mL	100 mL
Plate Covers, adhesive	2	20

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm, 490 nm, and 650 nm (polychromatic reading)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at thermofisher.com.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- For small-volume tubes, make sure to centrifuge the tubes before use to ensure pellets are not adhered to the cap.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.

Prepare 1X Wash Buffer

1. Dilute 25 mL of Wash Buffer Concentrate (20X) with 475 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 30 days.

Prepare 1X Assay Buffer

1. Dilute 5 mL of Assay Buffer (20X) with 95 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store 1X Assay Buffer at 2–8°C. The diluted buffer is stable for 30 days.

Pre-dilute samples

Due to the high sensitivity of the test, samples must be diluted prior to the 1:10 dilution on the plate. The following dilution factors are recommended as a starting point. Because the SARS-CoV-2 Ig titers can be very high, each investigator should determine the optimal dilution depending on whether a qualitative or quantitative assay is being performed.

Use a qualitative assay when trying to determine the absense or presence of antibodies. Use a quantitative assay if it is necessary to determine the titer levels of the antibody.

- Perform sample dilutions with 1X Assay Buffer.
- For qualitative assays, dilute serum and plasma samples 1:100 with 1X Assay Buffer to achieve the highest sensitivity.
- For quantitative assays, dilute serum and plasma samples 1:7,500 with 1X Assay Buffer if using a standard curve for samples with high Ig titer.

Reconstitute controls

High, Calibrator (Medium), and Low Controls can be used in the assay.

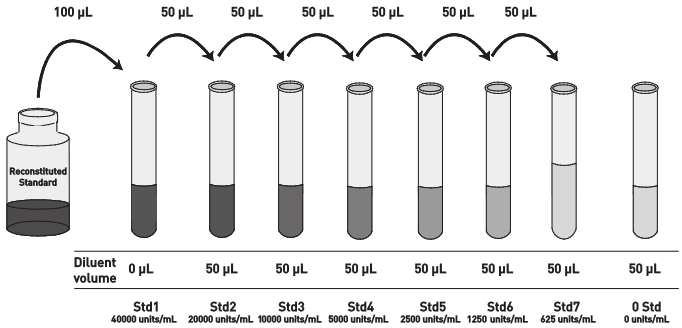
1. Reconstitute controls. See control vial labels for reconstitution volumes.
   a. Reconstitute Human SARS-CoV-2 Ig total High Control to 40,000 units/mL with deionized or distilled water.
   b. Reconstitute Human SARS-CoV-2 Ig total Calibrator to 10,000 units/mL with deionized or distilled water.
   c. Reconstitute Human SARS-CoV-2 Ig total Low Control to 2,500 units/mL with deionized or distilled water.
2. Swirl or mix gently, then incubate for 10 minutes. **Use the controls within 15 minutes of reconstitution**.

(*Optional*) Dilute standards for quantitative assay

For relative quantification, prepare a 2-fold serial dilution of the High Control to generate a standard curve.

Note: Use glass or plastic tubes for diluting standards.

1. Add 100 µL of reconstituted High control to a tube (Std1).
2. Add 50 µL 1X Assay Buffer to each of 7 tubes (Std2–Std7, 0 Std).
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded after completing the assay.



Prepare 1X HRP conjugate solution

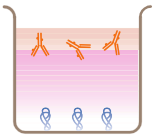
Note: Prepare 1X HRP conjugate solution within 15 minutes of usage.

For each coated plate, pipet 120 µL HRP Conjugate (100X), and dispense the solution into a tube containing 11.88 mL of 1X Assay Buffer. Mix thoroughly.

Perform Assay (Total assay time: 1.5 hours)

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- **Do not** perform this assay on a shaker.

1 Bind antibody

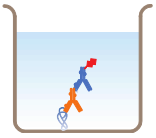


1.1. Wash wells 2 times with 1X Wash Buffer.
1.2. Add 90 µL of 1X Assay Buffer to all wells except the wells for blanks.
1.3. Add 10 µL of standards, controls, or samples (see “Pre-dilute samples” on page 1) to the appropriate wells.
1.4. Add 100 µL of 1X Assay Buffer to wells for blanks.
1.5. Cover the plate with a plate cover and incubate for 30 minutes at 37°C. **Do not shake**.
1.6. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer. **Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration**.

2 Add 1X HRP Conjugate

2.1. Add 100 µL 1X HRP Conjugate solution into each well.
2.2. Cover the plate with plate cover and incubate for 30 minutes at 37°C. **Do not shake**.
2.3. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer. **Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration**.

3 Add Substrate Solution



3.1. Add 100 µL Substrate Solution to each well. The substrate solution begins to turn blue.
3.2. Incubate for 15 minutes at room temperature.

Note: TMB should not touch aluminum foil or other metals.

4 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and analyze results

1. Read the absorbance at 450 nm. Read the plate immediately after adding the Stop Solution.
2. Determine the results of the assay.

Qualitative assay	Quantitative assay
Calculate the ratio of values using the equation: $Ratio = \frac{Absorbance\ Sample}{Absorbance\ Calibrator}$ • If the ratio is <1, the target Ig is absent. • If the ratio is 1–1.3, the result is indeterminate. • If the ratio is >1.3, the target Ig is present. • Divide the absorbance of the Low Control by the absorbance of the Calibrator as a negative control (ratio <1). • Divide the absorbance of the High Control by the absorbance of the Calibrator as a positive control (ratio >1.3).	1. If the High Control was used to generate a standard curve, use curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting. 2. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution. **Note:** Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor. Samples are diluted on the plate 1:10 to a final dilution of 1:75,000 for quantitative assays.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 4,000 units/mL Hu SARS-CoV-2 Ig total.

Standard High Control (units/mL)	Optical Density (450 nm)
4,000	2.79
2,000	1.72
1,000	0.97
500	0.59
250	0.34
125	0.18
62.5	0.11
0	0.02
Typical values for qualitative assays	
Calibrator	1.04
High Control	2.79
Low Control	0.34

Sensitivity

The limit of detection of human SARS-CoV-2 Ig total defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 28.96 units/mL (mean of 3 independent assays).

Reproducibility

Intra-assay precision

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with six replicates of serum samples containing different concentrations of human SARS-CoV-2 Ig total. Two standard curves were run on each plate. The following data show the mean human SARS-CoV-2 Ig total concentration and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 6.1%.

Sample	Experiment no.	Mean concentration (kU/mL)	% Coefficient of Variation
1	1	5215.6	4.0
	2	5122.8	4.0
	3	4726.6	5.3
2	1	6685.4	0.5
	2	6569.7	0.4
	3	6215.6	0.6
3	1	3460.6	11.1
	2	3344.6	10.0
	3	3134.3	5.5
4	1	6299.8	1.6
	2	6353.0	0.7
	3	5960.4	1.1
5	1	145.3	5.3
	2	125.5	11.2
	3	121.5	15.2
6	1	565.9	18.3
	2	516.6	3.5
	3	443.3	6.3
7	1	1349.7	10.7
	2	1193.9	6.5
	3	1314.1	6.0
8	1	473.2	8.6
	2	389.4	3.1
	3	350.9	6.2

Inter-assay precision

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with six replicates of serum samples containing different concentrations of human SARS-CoV-2 Ig total. Two standard curves were run on each plate. Data below show the mean human SARS-CoV-2 Ig total concentration and the coefficient of variation calculated on 18

determinations of each sample. The calculated overall inter-assay coefficient of variation was 7.6%.

Sample	Mean (kU/mL)	% Coefficient of Variation
1	5021.7	5.2
2	6490.3	3.8
3	3313.2	5.0
4	6204.4	3.4
5	130.8	9.7
6	508.6	12.1
7	1285.9	6.4
8	404.5	15.5

Recovery

Spike recovery was evaluated by spiking three different levels of human SARS-CoV-2 Ig total into serum and plasma (EDTA, heparin, citrate). Recoveries were determined with two replicates each. The amount of endogenous human SARS-CoV-2 Ig total in unspiked samples was subtracted from the spike values.

Sample	High spike (mean %)	Medium spike (mean %)	Low spike (mean %)
Serum	90	90	88
Plasma (EDTA)	91	91	87
Plasma (citrate)	89	94	93
Plasma (heparin)	90	94	89

Parallelism

Serum and plasma (EDTA, citrate, heparin) samples spiked with different levels of human SARS-CoV-2 Ig total were analysed at 2-fold serial dilutions with two replicates each.

Sample	Dilution	Expected recovery (mean %)
Serum	1:2000	101
	1:4000	100
	1:8000	98
Plasma (EDTA)	1:2000	105
	1:4000	103
	1:8000	101
Plasma (citrate)	1:2000	106
	1:4000	105
	1:8000	102
Plasma (heparin)	1:2000	104
	1:4000	100
	1:8000	107
SARS-CoV-2 positive sample	1:2000	104
	1:4000	105
	1:8000	105

Sample stability

Freeze-thaw stability

Serum samples were stored at –20°C and subjected to three freeze-thaw cycles, after which the human SARS-CoV-2 Ig total levels were determined. There was no significant loss of human SARS-CoV-2 Ig total immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum samples (spiked or unspiked) were stored at –20°C, 2–8°C, or room temperature (RT) for 24 hours, after which the human SARS-CoV-2 Ig total levels were determined. There was no significant loss of SARS-CoV-2 Ig total immunoreactivity detected during storage under the conditions that were tested.

Limited product warranty

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